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16. ABSTRACT

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Effects of environmental conditions on isoprene emission from live oak

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Abstract. Live-oak plants (*Quercus virginiana* Mill.) were subjected to various levels of CO_2, water stress or photosynthetic photon flux density to test the hypothesis that isoprene biosynthesis occurred only under conditions of restricted CO_2 availability. Isoprene emission increases as the ambient CO_2 concentration decreased, independent of the amount of time that plants had photosynthesized at ambient CO_2 levels. When plants were water-stressed over a 4-d period photosynthesis and leaf conductance decreased 98 and 94%, respectively, while isoprene emissions remained constant. Significant isoprene emissions occurred when plants were saturated with CO_2, i.e., below the light compensation level for net photosynthesis (100 μmol m^{-2} s^{-1}). Isoprene emission rates increased with photosynthetic photon flux density and at 25 and 50 μmol m^{-2} s^{-1} were 7 and 18 times greater than emissions in the dark. These data indicate that isoprene is a normal plant metabolite and not – as has been suggested – formed exclusively in response to restricted CO_2 or various stresses.

Key words: Carbon dioxide and isoprene emission – Isoprene – Light and isoprene emission – *Quercus* – Water stress.

Introduction

Isoprene (2-methyl-1,3-butadiene) has been identified as a volatile emission product from numerous plant species (e.g., Rasmussen 1970; Sanadze and Kursanov 1966). Its biosynthesis and emission were identified as light-dependent processes that increased with light intensity (Rasmussen and Jones 1973; Sanadze and Kalandadze 1966; Sanadze and Kursanov 1966; Tingey et al. 1979). Emissions also increased sharply with increasing temperature, reaching maxima between 40 and 43° C depending upon the species, followed by a sharp decline at higher temperatures (Sanadze and Kalandadze 1966; Rasmussen and Jones 1973; Tingey et al. 1979).

Isoprene production was shown to be associated with photosynthesis since isoprene biosynthesis occurred within the chloroplasts and isotopically labeled CO_2 was rapidly incorporated into isoprene (Sanadze et al. 1972; Mgaloblishvili et al. 1979). Both isoprene emission and photosynthesis increased with light intensity and the processes were light-saturated at similar intensities (Sanadze and Kalandadze 1966). Isoprene production also increased as the ambient CO_2 concentration decreased (Jones and Rasmussen 1975). Sanadze (1969) reported that isoprene production occurred only when CO_2 had decreased below the level that saturated photosynthesis. Emissions also increased as the light compensation point was reached. These observations led Sanadze (1969) to suggest that isoprene biosynthesis occurred only under conditions of restricted CO_2 availability.

We have carried out experiments with live oak in which the CO_2 concentration, plant water status, and photosynthetic photon fluence density (PPFD) were varied to determine: 1) the association between isoprene emissions and leaf conductance, 2) the association of isoprene synthesis and net photosynthesis, and 3) if isoprene was also synthesized under conditions of CO_2 saturation.

Materials and methods

Plant culture. Live oak (*Quercus virginiana* Mill.) seedlings were obtained from the Division of Forestry, Florida Department of Agriculture and Consumers Services, Tallahassee, USA. The plants were cultured in a greenhouse at maximum day/night temperatures of 23/18° C; sunlight was supplemented and the photoperiod extended to 16 h/day with high-intensity-discharge sodium-vapor lamps (Tingey et al. 1979). At least four weeks before sampling, plants were placed in a growth chamber and conditioned at day/

Abbreviation: PPFD = photosynthetic photon flux density

night temperatures of 27/18° C with 16-h photoperiods at a PPFD of 400 μmol m^{-2} s^{-1} provided by a mixture of cool-white fluorescent (3800 W) and incandescent (670 W) lamps. At the time of experimentation, the plants were approximately three years old and had both young and mature leaves.

Gas-exchange chamber. A dynamic mass-balance gas-exchange chamber, which functioned as a constant stirred tank reactor producing uniform conditions throughout the chamber, was used to determine isoprene emission rates (Tingey et al. 1979). The chamber was housed within a controlled environment chamber which provided cooling; the output of a high-intensity-discharge sodium-vapor lamp (Lucalox HPS, 1000 W; General Electric Co., Cleveland, O., USA) was controlled with neutral density filters to vary PPFD. Ambient air was pumped through an Aadco pure air generator (Aadco, Rockville, M., USA) to remove hydrocarbons, CO_2 and water vapor. Water vapor and CO_2 were added back to the airstream to obtain the desired concentrations within the gas-exchange chamber. Water vapor, CO_2 and isoprene were measured at the gas-exchange chamber inlet and outlet ports, and airflow was measured at the chamber inlet. During experimentation, the CO_2 concentration within the gas-exchange chamber was maintained at 350 ± 20 μl l^{-1}. The dew point of the air entering the chamber was constant during an experiment at approx. 0° C, whereas the dew point of the air exiting the chamber varied between 5 and 20° C depending on the light and temperature conditions of a given experiment. Air temperature was measured with a ventilated and shielded thermocouple, leaf temperature with a thermocouple pressed against the abaxial leaf surface in midcanopy. Photosynthetic photon fluence density at canopy height was measured with a Li-Cor Instruments Model LI-190 SR Quantom Sensor (Li-Cor, Lincoln, Neb. USA).

Isoprene emission, photosynthetic and transpiration rates were determined as follows.

$$J_x = C_x F A^{-1}$$

where J_x = net flux rate for isoprene, CO_2 or water vapor; C_x = plant-induced change in the concentration of the gas as it passed through the chamber; F = air flow rate through the chamber; A = leaf area. Leaf conductance to isoprene was calculated as follows:

$$L_c = J_t\, Dr$$

where L_c = leaf conductance for isoprene; J_t = transpiration rate; D = vapor pressure deficit between the leaf interior and the ambient air; r = ratio of isoprene diffusivity to water vapor diffusivity.

Experimental protocol. To determine if isoprene emissions were associated with the stomata, small glass cuvettes (diameter, 2 cm; volume, 25 ml) were clipped to the adaxial and abaxial leaf surfaces of attached leaves so that the epidermis did not exhibit visual damage. The cuvettes remained on the leaves for 4 h, when a 1-ml gas sample was withdrawn through a septum and assayed for isoprene. Subsequently, epidermal imprints of the adaxial and abaxial leaf surfaces were made (Williams 1973) to determine stomatal frequency.

To determine the relationship between CO_2 concentration and isoprene production, plants were maintained under constant conditions of PPFD (550 μmol m^{-2} s^{-1}), leaf temperature (29° C) and ambient CO_2 concentration (350 μl l^{-1}), for 7.5 h, before the CO_2 flow to the chamber was stopped. Within 25 min, the CO_2 concentration within the chamber decreased to approx. 40–55 μl l^{-1}. This concentration was maintained for the duration of the study by CO_2 evolution from the plant.

Additional studies were conducted to determine if the amount of time that plants photosynthesized under ambient CO_2 levels influenced the amount of isoprene released when plants were in a low CO_2 environment. In these studies the CO_2 was eliminated from the incoming air 1 h before, or 0, 1, and 2 h following the initiation of the light period. In all studies, isoprene was sampled at 10 min intervals both before and after CO_2 flow into the chamber was discontinued. In both CO_2-depletion studies, the plants were maintained in the dark for 8 h prior to receiving light.

The influence of soil moisture on isoprene emissions was measured over a 5-d period. One d prior to the start of the gas-exchange measurements, each plant was watered to field capacity, weighed, and placed in the gas-exchange chamber. The plants were not watered for the remainder of the experiment. Plants were maintained under the normal (16 h light 8 h dark) photoperiod. Isoprene emissions, net photosynthesis, transpiration rate, and leaf conductance were measured for the last 3 h of each light period and the average emission rate determined. Every 24 h, at the termination of the measurement period, the plant was removed from the chamber and weighed to determine the decrease in water content.

To establish the relationship between PPFD and isoprene production, each of four plants was maintained in the dark for at least 8 h. The PPFD was then increased in sequential fashion to a maximum of 150 μmol m^{-2} s^{-1}. After each incremental change in intensity, 1 h was allowed for equilibration at which time triplicate air samples were collected. Both isoprene emissions and CO_2 exchange were measured.

Isoprene sampling and analysis. Air samples (20 ml were collected from the sample ports of the gas-exchange chamber using a 100-ml gas-tight syringe, and cryogenically concentrated (Tingey et al. 1979). Isoprene was chromatographically separated and quantified as described in Tingey et al. (1979) except that a K_2CO_3 precolumn (9 cm long, 0.5 mm inner diameter) was used to remove water from the sample. The gas-chromatographic detector was standardized daily using three to six samples of 2,2,4-trimethylpentane (4.3 ng) (Scott-Marrin, Riverside, Cal., USA) with a reproducibility of ± 2%. A 99.9% pure isoprene standard (Aldrich Chemical Co., Milwaukee, Wis. USA) was used to establish the isoprene retention-time and profile. Positive identification of the isoprene emissions from live oak was made by a combination of gas chromatography and mass spectrometry (Tingey et al. 1979).

Data analysis. The relationship between means and standard deviations for isoprene emission, transpiration, and leaf conductance indicated that the responses followed lognormal statistical distributions. Therefore, the data were transformed to the respective logarithms for statistical analyses (Snedecor and Cochran 1968). Analyses of the photosynthesis data were conducted in the original scale.

The studies of CO_2-concentration and water-stress effects on isoprene emissions were randomized block experiments, with plants as the blocking factor. In the CO_2-depletion experiments, each day was divided into two periods: 1) ambient CO_2 concentration, and 2) decreased CO_2 concentration. The average isoprene emission for each plant in each CO_2 condition was calculated as the response variable. The effects of CO_2 on isoprene emissions were then evaluated by analysis of variance. The effects of water stress on isoprene emissions and gas-exchange parameters were also evaluated by analysis of variance. In this case, the mean isoprene emissions, net photosynthesis, transpiration, and leaf conductance for each plant for each day were used as the response variables.

The relationship between PPFD and isoprene emission was best described by a rational function which was estimated using a nonlinear least-squares procedure (Marquardt 1970). The relationship between light intensity and CO_2 flux was described by a linear regression equation for each plant. The regression coefficients for the four plants were averaged to obtain the maximum-likelihood estimate of the straight line that best fit all four plants (Graybill 1976).

Table 1. Relationship between stomatal frequency and isoprene emission in live oak leaves. The geometric means are based on nine observations; the number in parenthese is the standard geometric deviation. Plants were incubated at 30° C and a PPFD of approx. 350 μmol m^{-2} s^{-1} in static cuvettes

Leaf surface	Stomatal frequency (No. mm^{-2})	Isoprene emission (ng C cm^{-2} $4h^{-1}$)
Adaxial	0	22.1 (2.13)
Abaxial	535.2 (1.22)	622.4 (2.18)

Results

Stomatal study. Live oak had no stomata on the adaxial leaf surface, but more than 500 stomata per 1 mm^2 on the abaxial surface (Table 1). Substantially more isoprene accumulated in the cuvettes on the abaxial surface, approx. 96% of the total, indicating an association between isoprene emissions and stomata. In similar studies, isoprene accumulated above both leaf surfaces of *Plantanus occidentalis* L. which is an amphistomatic plant (data not shown).

CO_2 concentration. To determine the relationship between isoprene emissions and CO_2 concentration, plants were maintained under conditions of constant CO_2, PPFD (550 μmol m^{-2} s^{-1}) and leaf temperature (29° C) for approx. 7.5 h when the CO_2 concentration was reduced. Isoprene emissions averaged 12.8 μg C dm^{-2} h^{-1} at the ambient CO_2 concentration (350 μl l^{-1}); and 15.3 μg C dm^{-2} h^{-1} (standard geometric deviation, 1.19) at a reduced CO_2 concentration (40–55 μl l^{-1}). Emissions in the low CO_2 condition remained essentially constant for 4 h when measurements were terminated. Leaf conductance to isoprene increased concurrent with the increased isoprene emissions from 0.09 cm s^{-1} at the ambient CO_2 concentrations to approx. 0.13 cm s^{-1} at the low CO_2 level. The 44% increase in leaf conductance was associated with a 19% increase in isoprene emissions. The increased emissions with decreased CO_2 concentration were similar to those described in earlier reports (Sanadze 1969; Jones and Rasmussen 1975).

In previous studies, isotopically labeled CO_2 was readily incorporated into isoprene; the elevated isoprene emissions that occurred in the low CO_2 conditions decreased with the time that the plants were maintained at low CO_2 levels (Sanadze and Kursanov 1966; Sandze et al. 1972; Jones and Rasmussen 1975). These data indicated that a small, labile pool, closely associated with current photosynthesis, contributed to isoprene emissions. To test this hypothesis, the amount of time that plants photosynthesized at ambient CO_2 levels was varied as a means of altering photosynthetic pools. As in previous studies, isoprene emission rates increased significantly when the CO_2 concentration was decreased (Table 2); the elevated emissions were constant for the remaining 4 h of the study. Isoprene emission increased 45–55% and leaf conductance to isoprene increased 40–55% when the CO_2 concentrations decreased after the plants had photosynthesized for 1 or 2 h. Plants that concurrently experienced a reduced-CO_2 environment and received light, emitted a similar amount of isoprene as plants placed in a low-CO_2 environment after 1 or 2 h of photosynthesis. Plants that were maintained in a low-CO_2 environment for 1 h prior to receiving light emitted 84–88% as much isoprene in the light as plants that had photosynthesized for 1 or 2 h prior to CO_2 depletion.

Table 2. Effect of CO_2 concentration on isoprene emissions by live oak plants. Geometric means are based on two plants and the standard geometric deviation is 1.206. PPFD was 550 μmol m^{-2} s^{-1} and leaf temperature was 29 ± 1° C

Hours of light before CO_2 removal	Isoprene emission rate (μg C dm^{-2} h^{-1}) at.... μl l^{-1} CO_2	
	350	40–55
−1[a]	0.03	15.88
0[a]	0.05	18.36
1	11.47	17.81
2	12.87	18.54

[a] Isoprene emission rate data at 350 μl l^{-1} CO_2 were collected when the plants were in the dark. CO_2 addition to the chamber was stopped 1 h before and at the same time the lights were turned on for the −1- and 0-h treatments, respectively

Dark emissions. Isoprene emissions were detected from plants conditioned for 8 h in the dark (Table 2, Fig. 1A). Although emissions in the light were substantially larger, emissions in the dark were approx. 0.02–0.06 μg C dm^{-2} h^{-1}. Additional studies indicated that emissions from plants were similar after either 1 or 8 h in the dark.

Water-stress effects. To determine the influence of water stress on isoprene emission and gas-exchange parameters, plants were watered to field capacity approx. 24 h before the start of the experiment and received no additional water over a 5-d period (Table 3). At the end of the first measurement period, the water content of the plant-soil mass was 76% and it decreased to 24% on the fifth day. The isoprene emissions in the light remained essentially constant at approx. 14 μg C dm^{-2} h^{-1} during the first four days but decreased on the fifth day. In contrast to the relatively stable isoprene emissions, net photosynthetic rate, transpiration rate, and leaf conductance (Table 3) decreased daily from the first day, exhibiting

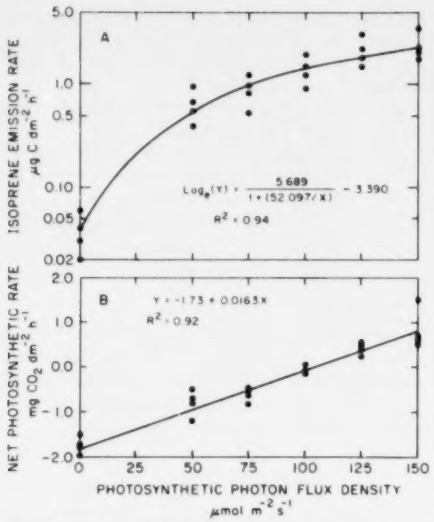

Fig. 1 A, B. The effect of varying light intensity on isoprene emissions (**A**) and CO_2 exchange (**B**) in live oak. Four plants were used to develop the curves and each plant was sampled at each PPFD. During experimentation leaf temperature was $29 \pm 1°$ C. Note that the ordinate scale in **A** is logarithmic

Table 3. Effect of water stress on isoprene emissions and other gas-exchange parameters in live oak plants. Means are geometric except for net photosynthetic rate and moisture content; all are based on four plants. SGD: standard geometric deviation; SD standard devition. The plants were on a 16-h photoperiod with a PPFD of 550 μmol m^{-2} s^{-1} and a leaf temperature of $29 \pm 1°$ C during the light

Days from watering	Moisture content (%)	Isoprene emission rate (μg C dm^{-2} h^{-1})	Net photosynthetic rate (mg CO_2 dm^{-2} h^{-1})	Transpiration rate (mg H_2O dm^{-2} h^{-1})	Leaf conductance (cm s^{-1})
1	76	14.0	4.80	398	0.093
2	53	13.9	3.97	373	0.079
3	36	14.9	1.22	224	0.024
4	26	14.0	0.09	131	0.008
5	24	7.0	−0.26	97	0.005
	SD 5.37	SGD 1.53	SD 1.54	SGD 1.31	SGD 2.24

approximately similar decreases as the decline in percent moisture content in the plant-soil mass. By the fourth day, there was essentially no net photosynthesis and on the fifth day respiration exceeded photosynthesis, yielding a net CO_2 release. Transpiration rates decreased from approx. 398 mg H_2O dm^{-2} h^{-1} on day 1 to 97 mg H_2O dm^{-2} h^{-1} on day 5. Similarly, leaf conductance for isoprene decreased from 0.35 on the first day to 0.02 cm s^{-1} by the end of the study.

Isoprene emissions at CO_2 saturation. To determine the association between PPFD and isoprene emissions, plants were placed in the dark for 8 h and then the light intensity was increased stepwise to 150 μmol m^{-2} s^{-1} (Fig. 1 A). Average emissions at 50 μmol m^{-2} s^{-1} (0.55 μg C dm^{-2} h^{-1}) were approx. 18 times larger than emissions in the dark (0.03 μg C dm^{-2} h^{-1}). In a subsequent study, isoprene emissions increased from 0.02 to 0.13 μg C dm^{-2} h^{-1} when the PPFD was increased from 0 to 25 μmol m^{-2} s^{-1}. As the the PPFD increased the amount of CO_2 emitted decreased (Fig. 1 B) and respiration was balanced by net CO_2 fixation at approx. 100 μmol m^{-2} s^{-1}. At higher PPFDs net carbon fixation occurred.

Discussion

Stomata are the major diffusive pathway for CO_2 used in photosynthesis; however, biochemical processes (mesophyll resistance) controlling CO_2 fixation are frequently the limiting factors, depending on the relative magnitudes of the mesophyll and stomatal components (Nobel 1974). Our data indicated that isoprene, similar to CO_2, diffused through the stomata with only a small proportion possibly diffusing through the cuticle. However, even though the stomata were the major diffusive pathway they did not seem to be the major factor controlling isoprene emissions from the leaf. In the water-stress study, leaf conductance decreased approximately an order of magnitude over a 4-d period (Table 3) but isoprene emission remained constant, indicating that large changes in stomatal conductance did not influence emission rates. When the CO_2 concentration was decreased leaf conductance rose 44% while isoprene emissions increased only 19%, indicating that an internal factor controlled emission as has been shown for CO_2. The indication that isoprene emission rates were controlled by an internal factor was supported by the observations of the Q_{10} of emissions over the range of 20–34° C (Rasmussen and Jones 1973; Sanadze and Kalandadze 1966; Tingey et al. 1979). The calculated Q_{10}'s ranged between approx. 2 and 4, indicating the enzymatic nature of isoprene synthesis. Based upon the rate-limiting-reaction concept, the Q_{10}'s may be used in determining the limiting step in a series of reactions (Giese 1973). The magnitude of the Q_{10}'s (2–4) indicated that isoprene biosynthesis or pool size was a more important factor in controlling emissions than stomata. Using different experimental approaches, Jones and Rasmussen (1975) also concluded that isoprene emissions were regulated by internal factors (isoprene synthesis) rather than stomatal conductance.

Field estimates of isoprene emissions from several oak (Zimmerman 1979) and other hardwood tree species (Flyckt et al. 1979) varied from similar to approx. 2.5 times greater than the emission rates reported in the current study. The higher field emission rates may reflect either species differences in emission rates and/or higher PPFD's in the field than used in this study. However, the measured isoprene emission rates in this study were similar to previous emission rates for live oak made at similar PPFD's (Tingey et al. 1979). Studies with *Populus nigra* (Sanadze 1969) and live oak (Tingey et al. 1979) indicated that approx. 1–2% of the photosynthetically fixed carbon was emitted as isoprene. Similar carbon-loss ratios were found in the current study, except when water stress caused a major reduction in photosynthesis; then the porportion of carbon lost as isoprene to carbon fixed increased dramatically. Even though the proportion of carbon lost increased, the absolute isoprene emissions did not increase.

Isoprene emissions were reported to be a light-dependent process; when plants were placed in the dark, evolution rapidly ceased (Sandze and Kalandadze 1966; Sanadze and Kursanov 1966; Rasmussen and Jones 1973). The rapid decrease in isoprene emissions in the dark indicated that isoprene pools were rapidly depleted by volatization. This observation was supported by the failure to identify isoprene in plant extracts (see Bonner 1965). In our studies, isoprene emissions also decreased rapidly when the plants were in the dark, indicating a small isoprene pool in live oak. However, emissions after 1 h in the dark were similar to emissions after 8 h in the dark (0.02 to 0.06 μg C dm^{-2} h^{-1}), indicating that the isoprene pool was either slowly depleted or that isoprene was continuously synthesized from an intermediate in dark.

Plants rapidly incorporate isotopically labeled CO_2 into isoprene (Sanadze 1969; Sanadze et al. 1972). Although isoprene may contain carbon fixed during current net photosynthesis, our data indicate that isoprene emission is not closely linked to current net photosynthesis. When plants were at a low CO_2 concentration (40–50 μl l^{-1}) for 4 h isoprene emissions remained constant over the period. Plants that were in the dark and at a reduced CO_2 level before light was present, emitted isoprene in a low CO_2 concentration in the light at approximately the same rate as plants that had photosynthesized for 1 or 2 h before the CO_2 concentration was reduced (Table 2). Plants that were water stressed (days 4 and 5) emitted appreciable amounts of isoprene although net photosynthesis did not occur (Table 3). The lack of correlation between net photosynthesis and isoprene emissions could indicate that the CO_2 levels within the leaf were sufficient to meet the carbon requirements of isoprene biosynthesis or that isoprene was synthesized from intermediates distantly related to the primary products of carbon fixation.

The emission of isoprene during prolonged dark periods and the lack of close correlation between net carbon fixation and isoprene emissions indicate that isoprene may be synthesized from intermediates not closely related to carbon fixation. Jones and Rasmussen (1975) reported that leaf discs incubated with either C^{14}-labeled glycine or glycolate rapidly incorporated the label into isoprene and that inhibitors of the photorespiration cycle dramatically reduced isoprene biosynthesis. They suggested an association between isoprene and β-carotene biosynthesis. More recently, Loomis and Croteau (1980) interpreted these and other data as indicating an association between photorespiration and isoprene biosynthesis. If isoprene was produced from intermediates of the photorespiration cycle, this would explain the observations in our studies.

Sanadze (1969) suggested that isoprene emissions occurred only when CO_2 was not saturating for photosynthesis. The increased isoprene emissions observed when the CO_2 concentration was reduced supported this hypothesis. However, substantial isoprene emissions occurred at PPFD below that required for net photosynthesis (Fig. 1 A). At these intensities, respiration exceeded photosynthesis and the plants emitted CO_2 indicating CO_2 saturation. These data indicate that isoprene is a normal plant metabolite and not only formed when plants were CO_2 deficient.

References

Bonner, J. (1965) The isoprenoids. In: Plant Biochemistry, pp. 665–692. Bonner, J., Varner, J.E. eds. Academic Press, New York

Flyckt, D.L., Westberg, H.H., Holdren, M.W. (1980) Natural organic emissions and their impact on air quality. Reprint 80-69.2, Ann. Meet. Air Pollution Control Ass., 22–27 June, 1980, Montreal, Canada

Giese, A.C. (1973) Cell physiology, 4th edn. W.B. Saunders Co., Philadelphia, Pa., USA

Graybill, F.A. (1976) Theory and application of the linear model. Duxbury Press, North Scituate, Mass, USA

Jones, C.A., Rasmussen, R.A. (1975) Production of isoprene by leaf tissue. Plant Physiol. **55**, 982–987

Loomis, W.D., Croteau, R. (1980) Biochemistry of terpenoids. In: The biochemistry of plants, vol. 4, pp. 363–415 Stumpf, P.K., Conn, E.E., eds. Academic Press, New York

Marquardt, D.W. (1970) Generalized inverses, ridge regression, baised linear estimation, and nonlinear estimation. Technometrics **12**, 591–612

Mgaloblishvili, M.P., Khetsuriani, N.D., Kalandadze, A.N., Sanadze GA (1979) Localization of isoprene biosynthesis in poplar chloroplasts. Sov. Plant Physiol. **25**, 837–842

Nobel, P.S. (1974) Biophysical plant physiology. W.H. Freeman and Co., San Francisco

Rasmussen, R.A. (1970) Isoprene: Identified as a forest-type emission to the atmosphere. Environ. Sci. Technol. **4**, 667–671

Rasmussen, R.A., Jones, C.A. (1973) Emission of isoprene from leaf discs of *Hamamelis*. Phytochemistry 12, 15–19

Sanadze G.A. (1969) Light-dependent excretion of molecular isoprene by leaves. Prog. Photosynth. Res. **2**, 701–706

Sanadze, G.A., Kalandadze, A.N. (1966) Light and temperature curves of the evolution of C_5H_8. Sov. Plant Physiol. **13**, 411–413

Sanadze, G.A., Kursanov, A.L. (1966) On certain conditions of evolution of the diene C_5H_8 from poplar leaves. Sov. Plant Physiol. **13**, 184–189

Sanadze, G.A., Dzhaini, G.I., Tevzadze, T.M. (1972) Incorporation into the isoprene molecule of carbon from $C^{13}O_2$ assimilated during photosynthesis. Sov. Plant Physiol. **19**, 17–20

Snedecor, G.W., Cochran, W.G. (1968) Statistical methods, 6th edn. Iowa State Univ. Press, Ames, USA

Tingey, D.T., Manning, M., Grothaus, L.C., Burns, W.F. (1979) The influence of light and temperature on isoprene emission rates from live oak. Physiol. Plant. **47**, 112–118

Williams, J.A. (1973) A considerably improved method for preparing plastic epidermal imprints. Bot. Gaz. **134**, 87–91

Zimmerman, P.R. (1979) Determination of emission rates of hydrocarbons from indigenous vegetation in the Tampa/St. Petersburg, Florida area. In: Appendix C, Tampa Bay area photochemical oxidant study. EPA-904/9-77-028. U.S. Environmental Protection Agency, Region IV, Atlanta, Ga.

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